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STRUCTURE: FUNCTION RELATIONSHIPS IN THE GLUTATHIONE TRANSFERASES

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The relationship between catalytic prowess and the structure of the cytosolic glutathione S-transferases as revealed by regional and site-specific mutagenesis and the physical techniques of NMR spectroscopy and x-ray crystallography will be discussed. The interplay of the molecular biological manipulation of the gene and gene products with the physical and catalytic characterization of native and altered enzymes will be emphasized in an attempt to elucidate the influence of the active site structure on the catalytic properties of the class mu isoenzymes.

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ACTIVE SITE-DIRECTED INHIBITION

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Selective inhibitors of cytochrome P450 dependent activities have been used in attempts to distinguish catalysis by various P450 isozymes. The inhibition of P450 dependent catalysis is influenced by a combination of hydrophobic, electronic, and stereochemical effects and compounds that bind reversibly and function as either alternate substrates or as ligand binding reagents provide limited information concerning details of the active-sties of different P450 isozymes. Mechanism-based irreversible inhibitors (suicide inhibitors) of P450 dependent enzymes have been developed which do provide useful information about the active-site regions of the P450 isozymes. Since suicide inhibitors must bind within the P450 active-site with a potentially reactive functional group in juxtaposition to the perferryl oxygen, suicide inhibition implies that a given molecule is complementary to the active-site. Furthermore, as demonstrated by Ortiz de Montellano and his coworkers, when suicide inhibitors act by binding irreversibly to groups within the active-site the resulting products can be used to deduce features of active-site geometry. Experiments utilizing substituted acetylenes as suicide inhibitors of P450 isozymes demonstrate that this class of compound may be used both to differentiate among active-sties of P450 isozymes and to establish structural features of particular P450 active-sites. Since metabolic activation of acetylenes by P450 catalysis depends critically on the relative positioning of the carbon-to-carbon triple bond and the perferryl oxygen, suicide inhibition of P450 isozymes by acetylenes is critically dependent upon complementary binding within the active-site. Recent results indicate acetylenes can be employed *in vivo* as selective suicide inhibitors of the particular P450 isozymes that are responsible for the activation of chemical procarcinogens. Some of the research described has been supported by grant CA 38192 from the USPHS.

BIOACTIVATION AND TOXICITY - IMPLICATIONS FOR DRUG DESIGN

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An important principle which has emerged from mechanistic studies in biochemical toxicology is that many drugs, industrial chemicals, pesticides and pollutants are not toxic *per se*, but elicit their adverse effects only after metabolism to chemically-reactive intermediates which interact with endogenous components of the host cell. These reactive intermediates, which may be produced by oxidation, reduction or conjugation processes, are electrophilic in nature and bind covalently to a variety of cellular constituents including proteins and nucleic acids. In some cases, the consequences of such xenobiotic-macromolecule interactions are lethal to the host, in that they may result in cellular necrosis or the initiation of neoplastic states. In other cases, however, the consequences are less dramatic. For example, the enzymes which catalyze the formation of reactive metabolites may themselves serve as targets for either covalent modification or metabolic complex formation, resulting in irreversible and reversible enzyme inhibition, respectively. Such phenomena represent the basis for several known drug-drug interactions where clearance of a second drug is impaired as a result of inhibition of a key enzyme (e.g. cytochrome P-450) by the first drug.

Whatever the nature of the adverse effect associated with a particular foreign compound, detailed information on the metabolic fate of that agent can often be of considerable practical value in providing "leads" for the rational design of new drugs with improved therapeutic indices. In this presentation, examples will be given of two compounds which undergo metabolism to chemically-reactive intermediates, and where an understanding of the responsible metabolic pathways is being exploited in order to search for analogs with decreased toxicity. The drugs which will be discussed are *N*-methylformamide, an experimental antineoplastic agent which causes liver toxicity in both animals and human subjects, and stiripentol, a new antiepileptic drug which inhibits cytochrome P-450 activity and thereby causes a variety of drug-drug interactions.

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FREE RADICALS: EFFECTS AND PROTECTION MECHANISMS

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Currently, it is recognized that free radicals play a role in the physiology and pathogenesis. It has been suggested that the early involvement of free radicals in the evolution of life explains their ubiquitous presence and vital physiological role. The term oxygen free radicals is generally used more or less synonymously with reactive oxygen species (ROS).

ROS are involved in biotransformation of xenobiotics, in the regulation of contraction of smooth muscle and in the phagocytic cell function. Damage inflicted by ROS may affect polyunsaturated fatty acids (lipid peroxidation), DNA, proteins and carbohydrates. In conjunction with the omnipresent ROS generation, the organism has been equipped with a complex armory of effective defence systems in order to prevent ROS damage to cell constistuents. The structural characteristics of the cell membrane form a defence in itself. Penetration of ROS into the hydrophobic layer -where initiation of lipid peroxidation takes place- will not occur readily. A classification in defence systems can be made according to the level at which they interfere with the lipid peroxidation process.

The first line of defence includes the factors that prohibit the formation of, or scavenging of primary initiators of the lipid peroxidation process, like metal-iron-binding proteins, or the enzymes superoxide dismutase, catalase and selenium-dependent glutathione (GSH) peroxidase. The vitamins E and C as well as β-carotene may scavenge ROS and can therefore be classified as belonging to the first line of defence. However, particularly vitamin E, but also vitamin C can be regarded as chain-breaking antioxidants. Also bilirubine is a prominant chain-breaking antioxidant (second line defence). The metabolism of lipid peroxidation products forms the third line of defence. This type of defence can also be recognized in the process of oxidative damage to nucleic acids. Proteolytic activity is stimulated by preceding ROS mediated protein abberations. There is an interplay between various defence systems. Novel antioxidant drugs are now being developed.

SPECIES DIFFERENCES IN PROTEIN BINDING

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For most drugs, a systematic comparison of protein binding between species is lacking. However, conclusions based on comparison of results from different studies, can be misleading. Indeed, the percentage binding obtained depends on a number of experimental conditions, e.g. the method used and the concentration of the drug.

Species differences in the degree of binding to plasma proteins have been found for a number of drugs. Acidic drugs, which are mainly bound to albumin, are in general more extensively bound to serum in human beings than in other mammalian species. Nevertheless, the extent of binding in mammalian species is for most drugs within a range which permits a general classification of the binding as high, moderate or low. The observed differences may reflect different binding affinities as well as differences in albumin concentration.

For drugs mainly bound to AAG such as oxprenolol, propranolol and disopyramide, the differences in extent of binding between species are more pronounced but data in the literature are scarce.

Enantioselectivity of binding to serum proteins can be species-dependent, both for albumin and for AAG.

Protein binding plays a major role in the distribution and elimination of drugs and some of the differences in the pharmacokinetics among species, can be explained by differences in protein binding.

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TRANSGENIC ANIMALS

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In the early 1980s, exogenous genetic material was first introduced into mouse embryos leading to stable integration of the foreign DNA and subsequent propagation of transgenic animals. Today this technology has become a key research tool in understanding the control and regulation of gene expression higher organisms. More recently this technology has been applied to the discovery of new biologically active molecules and for the understanding of how these processes can be altered either through drugs or by gene therapy. This brief session will:

1. Outline some of the problems in establishing a transgenic rodent model for pharmacological research. Specific emphasis will be place on the development of the MMTV-v-Ha-ras (Sinn, et al., Cell 49:465 1987) and other oncogene-containing transgenic mouse models.
2. Discuss the general principles used to solve some of the problems associated with developing a breeding and characterization program. In particular, the amount and kinds of effort involved in fully characterizing a transgenic model will be discussed.
3. Present several recent applications of transgenic animals to the drug discovery and evaluation processes. Studies of chemopreventive agents and dietary effects on cancer development in the transgenic animals will be shown.

ADDUCTS OF ETHYLENE OXIDE AND VINYL CHLORIDE AS INDICATORS OF EXPOSURE

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Ethylene oxide, a carcinogen in animals, is a major plastic monomer and sterilizing agent for heat sensitive materials. It is present in exhaust fumes, cigarette smoke and detergents manufactured on the basis of polyethylene glycol. In addition, it is formed metabolically in mammals from inhaled ethylene. Vinyl chloride, a proven human carcinogen, is also a major plastic monomer and can be formed by anaerobic bacteria in soil from the ubiquitous pollutants trichloroethylene and perchloroethylene.. This can lead to a contamination of drinking water by the substance.

Male Sprague-Dawley rats were exposed by inhalation to either ethylene oxide or vinyl chloride (3 doses/experiment; 3 animals/dose). The animals were sacrificed and liver DNA isolated by phenol extraction. The adducts 7-(2-hydroxoethyl)guanine in the case of ethylene oxide exposure and 7-(2-oxoethyl)guanine in the case of vinyl chloride exposure were subsequently determined by gas chromatography/mass spectrometry. The chemical binding indices (CBI) according to Lutz were calculated and compared with data published by other authors. A 70 fold higher genotoxic risk was thus found for vinyl chloride as compared to ethylene oxide.

In addition, 7-(2-hydroxoethyl)guanine was determined in lymphocyte DNA of animals and humans that had not been experimentally or occupationally exposed to ethylene oxide. A high adduct level was found, which supports the above mentioned exposure to the substance due to environmental pollution and endogenous production.

Finally, ^{14}C-labelled ethylene oxide was applied in vitro to human blood samples. The distribution pattern of radioactivity in the different blood compartments shows the influence of an enzyme polymorphism for ethylene oxide, which may modulate the formation of adducts.

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LACTATIONAL TRANSFER OF A LOW DOSE OF 3,3',4,4'-TETRACHLOROBIPHENYL (TCB) AND 2,2',4,4',5,5'-HEXACHLOROBIPHENYL (HCB) INDUCES CYTOCHROME P-450IVA1 IN NEONATES. EVIDENCE FOR A SYNERGISTIC MECHANISM

Jürgen T. Borlakoglu and Klaus D. Haegele, Marion Merrell Dow, Strasbourg, France

Lactational transfer of either TCB or HCB or a combination of both PCB-congener resulted in significant neonatal induction of total cytochrome P-450 and cytochrome b_5. By contrast, the N-demethylation of aminopyrine and dimethylnitrosamine was significantly reduced in response to TCB or combined treatment. This suggests a reduction of expressed catalytic activities of cytochrome P-450IIB. Treatment with TCB resulted in the induction of EROD activities, however, combined treatment with HCB and TCB resulted in significant increases in EROD activities, when compared with the TCB treatment alone. Enzyme activities normally associated with peroxisomal proliferation were marginally reduced in response to treatment with either TCB or HCB, however, combined treatment with HCB and TCB caused a highly significant reduction in peroxisomal β-oxidation and fatty acid Co A oxidation. The specific cytochrome P-450IVA1 concentration was significantly increased in response to HCB and TCB treatment, however, combined treatment with both PCB-congener resulted in a further increased ($p < 0.01$) expression of cytochrome P-450IVA1. This suggests a potential synergistic mechanism in response to a combined treatment. It is proposed that PCBs induce cytochrome P-450IVA1 by a mechanism that is likely to differ fundamentally to that observed by treatment with hypolipodaemic drugs.

OXIDATIVE METABOLISM IN VIVO

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Oxidative metabolism in man exhibits quite a variety of reactions, it involves several isozymes of the cytochrome P-450 family (and some other enzymes) and it shows a large degree of inter-subject variability in metabolic capacity. The latter issue has received major attention during the last decade, with the detection of the genetic polymorphism of the debrisoquine/sparteine type and the mephenytoin type. Furthermore a wealth of information became available on the various factors contributing to variability in the rate of drug metabolism through induction and inhibition and on the selectivity thereof with respect to the different isozymes involved.

Several probe drugs have been evaluated to assess oxidative drug metabolizing enzyme activity in vivo in man, such as antipyrine, aminopyrine, hexobarbital, theophylline, nifedipine. In order to obtain a more or less "complete" spectrum of isozyme activities, more than one probe drug is needed. By using a "cocktail" of suitable probes the existence of genetic polymorphisms, as well as the influence of factors and conditions affecting enzyme activity can be assessed in or two experimental sessions.*

*D.D.Breimer & J.Schellens, TiPs 11:223 (1990).

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TRENDS IN HPLC: ENHANCED SELECTIVITY AND SENSITIVITY VIA ON-LINE PRE- AND POST-COLUMN STRATEGIES

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A major part of modern analytical problem solving deals with the trace-level determination of organic constituents in complex biological and environmental samples by means of HPLC. In such cases, (on-line) sample pretreatment and/or post-column reaction detection - with, often, final detection via luminescence monitoring - are steps of great importance, because HPLC displays a separation efficiency and absolute detection sensitivity that are not fully comparable with those that can be obtained in capillary gas chromatography. In the present lecture, relevant aspects and novel trends of sample treatment and reaction detection will be discussed in some detail.

In the field of precolumn techniques, trace enrichment and sample clean-up via the use of short precolumns packed with non-selective hydrophobic stationary phases is now a routine procedure. Research areas of current interest include the use of metal-loaded sorbents, of precolumns packed with sorbents containing immobilized antibodies, and of on-line dialysis and electrodialysis. Increasing attention is being devoted to the use of on-line pre-column derivatisation reactions.

As regards post-column reaction detection, principles such as conventional derivatisation and ion-pair formation, photochemical reactions, heterogeneous catalysis or reaction, and chemiluminescence all are widely used in biomedical - as also in environmental - analysis. The proper construction of post-column reactors merits some attention, because undue band broadening should be avoided.

A selected number of the above aspects will be discussed and the potential of combining pre- and post-column strategies will be illustrated. In all instances, the practicality of the approaches will be demonstrated by including real-life examples about various types of samples (serum, urine, milk, eggs) and analytes (5-fluorouracil, AZT, nitrofurans, nortestosterone, clobazam, pheniramines, steroids). If time permits, some remarks will be made about a rather novel approach, on-line HPLC-capillary GC-MS.

SPECIES DIFFERENCES IN XENOBIOTIC METABOLISM: PUZZLES WORTH SOLVING!

John Caldwell
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Species differences in the metabolism of xenobiotics are of abiding interest to the drug metabolism scientist, being of both fundamental and practical significance. These represent major biological variables influencing metabolism and their study can give insight into the evolution and regulation of metabolism at the most basic level. These differences are also of major practical concern since variations in metabolism commonly underlie inter-species differences in both desired and undesired effects of chemicals on living organisms. The recognition and, increasingly, the understanding of the mechanistic basis of such species differences are essential for the objective extrapolation of animal data to humans and has become a cornerstone of the safety evaluation of drugs and a wide range of other chemicals to which the human population is accidentally or deliberately exposed.

Examples of fundamental studies on species differences and their practical exploitation will be drawn from current work in the author's laboratory, including interspecies variations in the stereochemistry of drug oxidation and reduction, the metabolism of glutathione conjugates and the role of species differences in metabolism in the safety assessment of food flavours and natural dietary toxins.

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EVIDENCE FOR COMPLEX FORMATION BETWEEN RABBIT LUNG FLAVIN-CONTAINING MONOOXYGENASE AND CALRETICULIN

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Rabbit lung flavin-containing monooxygenase (FMO, EC 1.14.13.8) was denatured, reduced, carboxymethylated, digested with endoproteinase Glu-C or trypsin and subjected to mass spectrometric analysis. The amino acid sequences of selected peptides were determined by tandem mass spectrometry. Over 90% of rabbit lung FMO was mapped by liquid secondary ion mass spectrometry. The FMO N-terminal amino acid was found to be N-acetylated, and the N-terminal 23 amino acid peptide contained an FAD binding domain consisting of Gly-X-Gly-X-X-Gly. Another peptide was found to contain a $NADP^+$ binding domain consisting of Gly-X-Gly-X-X-Ala. The peptide sequence was confirmed by tandem mass spectrometric sequencing of proteolytic peptides. The sequence was found to be identical to the peptide sequence deduced from the cDNA data and the previously published gas phase sequencing data.

Further mass spectrometry and protein analytical work unambiguously showed that rabbit lung FMO existed in tight association with a calcium-binding protein, calreticulin. Over 68% of rabbit lung calreticulin was mapped by LSIMS. Tandem mass spectrometric and gas phase sequencing studies provided direct evidence for the identification of the N-terminal and other rabbit lung calreticulin-derived peptide sequences that were identical to other previously reported calreticulins. The complexation of calreticulin to rabbit lung FMO could account for some of the unusual physical properties of this FMO enzyme form.

Supported by grants from the NIH (GM 36426 to J.R.C., HL 38650 to D.E.W., and RR 01614 to A.L. Burlingame, Director, UCSF Bioorganic Biomedical Mass Spectrometry Resource).

BIOACTIVATION AND PHYSIOLOGICAL MODELLING

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Biotransformation reactions often play a significant role in modulating the toxicity of xenobiotics. Relatively innocuous substances can be converted to highly toxic reactive metabolites. Conversely, toxic substances can be biotransformed to forms which are less reactive or more readily excreted, thus limiting the extent of their biological action. Evaluation of the dose-dependent toxicity of these compounds requires a measure of the time-dependent concentration of the toxic form in the target tissue. It is not sufficient to simply relate observed incidence of toxicity to exposure concentration or administered dose. The relationship between an external measure of dose and the internal "effective" dose can be greatly complicated by the interaction of such factors as capacity-limited biochemical processes, perfusion limitations, and induction or inhibition of enzyme activity. Pharmacokinetic models which are based on the actual physiological structure of the animal can incorporate these dose-dependent processes explicitly, providing a coherent description of seemingly complex behavior. In addition, their physiological structure makes them a valuable adjunct to experimental studies in elucidating mechanisms and kinetics of biotransformation and in describing the variation of these processes across species. This presentation will describe the process of developing a physiologically based pharmacokinetic model and the use of the model to investigate the biochemical processes which determine the kinetics and toxicity of a chemical. The application of the model to estimate human toxicity on the basis of animal studies and tissue assays will also be described.

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ENDOGENOUS AND EXOGENOUS SUBSTRATES FOR PULMONARY EPITHELIAL UPTAKE SYSTEMS

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The herbicide paraquat is selectively toxic to the lung. In the course of study of this selective toxicity, it was observed that paraquat was selectively accumulated in the lung by an energy and temperature dependent mechanism. The uptake of paraquat appears to be predominantly into alveolar epithelial type 1 and type 2 cells and Clara cells. A similar uptake system appears to be responsible for the accumulation of the endogenous oligoamines, putrescine, spermidine and spermine. More recently, we have shown that other endogenous molecules such as the disulphide cystamine are also accumulated by this uptake system. Following its accumulation, cystamine is rapidly metabolised to taurine, which may be an effective antioxidant or an inflammatory agent in the lung. In addition, the lung also possesses a separate uptake system for the accumulation of taurine. A similar but not identical uptake system for the accumulation of polyamines and structurally related molecules is also found in tumour tissue and may provide a basis for the design of selectively toxic anticancer agents.

ACKNOWLEDGEMENT: This work was supported in part from the Cancer Research Campaign of Great Britain.

TRANSPORT MECHANISMS IN THE KIDNEY.

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Renal excretion of drugs is the result of different mechanisms: glomerular filtration, passive back diffusion, binding tot he brush border of the proximal tubular cell followed by pinocytosis (fluid phase, carrier mediated), tubular secretion and tubular reabsorption. Of these mechanisms the last three are saturable as they involve carrier transport. This also implies that carried mediated pinocytosis, tubular secretion and tubular reabsorption are susceptible to competition between similar substrates for the common carrier site. Furthermore, transport via these mechanisms is energy dependent, it is so-called active transport able to concentrate a drug. Two examples of such transport mechanisms having important impact on their potential nephrotoxic effect will be discussed: renal handling of aminoglycosides and ß-lactam. Insights into the renal handling, mechanism of nephrotoxicity and the identification of risk factors enable the clinician to reduce drastically the prevalence and degree or renal damage induced by aminoglycosides and ß-lactams.

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The analysis of conjugates

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The structure elucidation of metabolites is at Organon performed by combination of mass spectrometry and NMR spectroscopy. The molecular mass and the nature of conjugated drug metabolites are usually unraveled by positive and negative FAB-MS. The stereochemical discrimination is normally done with NMR only. The use of NMR correlation spectra, ^{13}C NMR spectra, decoupling spectra and NOE-difference spectra will be illustrated with examples selected from current metabolism studies within Organon's research. With these techniques it was a.o. possible to determine the diastereoisomeric ratio of O-glucuronides, the formation of diastereoisomers as a consequence of a new chiral centre introduced by N-glucuronidation, the regioisomeric composition of acyl glucuronides and the discrimination between an O- or N-sulphated disubstituted aromatic compound. Examples of an uncommon conjugation with glycine and taurine of alkylcarboxylic metabolites will be shown. Furthermore, the structure elucidation of recently discovered carbomate glucuronides will be given.
The examples given show that the combination of NMR and MS techniques might result in the structure elucidation of well-known and unexpected new forms of conjugated metabolites.

INTERACTION OF DIFLUNISAL ACYL GLUCURONIDE AND ITS ISOMERS WITH ALBUMIN

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Acyl glucuronides are potentially reactive metabolites, capable of undergoing hydrolysis, intramolecular rearrangement (isomerisation via acyl migration) and intermolecular covalent bonding reactions to protein (i.e. drug-protein adduct formation). The mechanism of adduct formation is uncertain, but transacylation (with -SH, -OH, -NH_2 groups on protein) and Schiff's base formation (with -NH_2 groups of lysine residues) have been suggested. Acyl migration is a prerequisite for the latter mechanism.

Diflunisal acyl glucuronide (DAG) and its individual 2-, 3- and 4-*O*-acyl migration isomers were purified (preparative HPLC) from the urine of a volunteer who ingested diflunisal (DF). After incubation (at concentration *ca.* 55 µg DF equivalents/ml) with human serum albumin (HSA, 40 mg/ml) at pH 7.4 and 37° C, DF-HSA adduct formation was much greater from the isomers than from DAG itself e.g. adduct concentrations at 2 hr were 1.1, 4.9, 7.1 and 18.7 µg DF equivalents/ml for DAG and its 2-, 3- and 4-isomers, respectively. After incubation of DAG and an equimolar mixture of its three isomers in 1:1 (v/v) methanol/phosphate buffer pH 7.4 at 37° C, formation of DF methyl ester (i.e. a transacylation reaction) was 7 times greater from DAG than from its isomers after 10 min incubation. The results therefore do not support contention that transacylation constitutes the predominant mechanism of formation of covalent adducts of DF with HSA *in vitro*.

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GENETICALLY ENGINEERED V79 CELLS FOR DRUG METABOLISM STUDIES

Johannes Doehmer, Catherine Janssens, Michael Edigkaufer, Martina Barrenscheen, Karl Ludwig Platt, Hansruedi Glatt, Albrecht Seidel and Franz Oesch, Institut für Toxikologie, Universität Mainz, Germany

Mammalian cells are being genetically engineered for stable expression of xenobiotics metabolizing enzymes. This approach marks a fundamental and new development in the field of *in vitro* biotransformation models.
This laboratory concentrates on the development of V79 Chinese hamster derived cells genetically engineered for stable expression of cytochromes P450. V79 cells have been chosen to serve as recipient cell in cDNA mediated gene transfer for various reasons. V79 cells have been the preferred indicator cells in mutagenicity and cytotoxicity studies for almost twenty years. They are extremely well adapted to culture conditions, as they are among the fastest growing cell lines with an avarage doubling time of 12 hours, and as their karyotype is very stable. However, V79 Chinese hamster cells lack cytochrome P450 activity. As there is no endogenous P450 background activity, genetically engineered V79 cells are highly defined for one particular P450 isoform. This has already been demonstrated for rat cytochromes P450IIB1 (1), P450IA1 (2), and P450IA2 (3) in metabolism-, mutagenicity-, and cytotoxicity-studies. Recently, V79 cells were succesfully transfected with human cytochromes P450IA2 and IIE1 cDNA. (1):Doehmer et al., PNAS,vol.85,5769-5773, 1988; (2):Dogra et al.,Mol Pharmacol,vol.37,608-613, 1990; (3): Janssens et al., submitted;
Supported by Deutsche Forschungsgemeinschaft (SFB302) and Bundesgesundheitsamt (ZEBET)

HEMOGLOBIN AND DNA ADDUCTS

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The monitoring of human exposure to selected genotoxic carcinogens has been achieved by determinations of the amounts of covalently-bound products (adducts) that these compounds form with nucleic acids or proteins. The measurement of DNA adducts may be achieved using ^{32}P-postlabelling, immunological or physicochemical techniques (e.g. fluorescence, mass spectrometry). Sensitivities up to the detection of 1 modified base per 10^{10} bases may be achieved by ^{32}P-postlabelling. The protein most commonly used for carcinogen adduct determination is hemoglobin (Hb), for reasons of its ready availability and long life time in man (120 days). The most widely used analytical technique for Hb adduct determination is gas chromatography-mass spectrometry (GC-MS), although immunological techniques have also been developed. Recent developments in DNA and Hb adduct determination, with particular emphasis on GC-MS approaches, will be reviewed and selected examples from occupational, environmental and medicinal human exposure to genotoxic species will be presented.

For example, hydrolysis of DNA with which benzo(a)pyrene has formed an adduct (via its metabolic activation to the 7,8-dihydrodiol,9,10-epoxide) yields the 7,8,9,10 tetrol which may be extracted and analysed by fluorescence spectroscopy or GC-MS. Genotoxic activation of styrene is to the 7,8-epoxide which forms esters with carboxylic acids in Hb which may similarly be hydrolysed liberating styrene glycol which can be determined by GC-MS. Aromatic amine adducts with Hb include cysteine sulphinamides which are also susceptible to hydrolysis; the use of a method for determining occupational exposure to 4,4'-methylene dianiline will be demonstrated. A modified Edman degradation allows the quantitation of carcinogen adducts formed following exposure to alkylating agents, at the N-terminal position of Hb. We have employed this technique to monitor exposure to hydroxyethylating agents from cigarette smoke and anti-cancer chloroethylnitrosoureas, and occupational exposure to ethylene oxide and styrene oxide.

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